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SYSTEMATIC RELATIONSHIPS OF SARRACENIACEAE INFERRED FROM NUCLEAR RIBOSOMAL DNA SEQUENCES

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ABSTRACT

The Sarraceniaceae are a small family of insectivorous herbs native to North and South America. The family is composed of three geographically separated genera, *Heliamphora, Darlingtonia* and *Sarracenia*. Recent molecular evidence suggests that *Darlingtonia* is sister to a *Heliamphora-Sarracenia* clade. The systematic relationships among the taxa within the genus *Sarracenia* are uncertain. Within the *S. rubra* and *S. purpurea* complexes, five and four infraspecific taxa have been named respectively. In this study, combined Internally Transcribed Spacer 2 and 26S large ribosomal subunit rRNA gene DNA sequences were used to infer phylogenetic relationships among the genera within Sarraceniaceae and the specific and subspecific taxa within *Sarracenia*. Results from this study support the sister relationship between *Darlingtonia* and a *Sarracenia-Heliamphora* clade. Within the genus *Sarracenia, S. purpurea* is sister to all remaining species. Additionally, the four named infraspecific taxa of *S. purpurea* are resolved in a well-supported clade. However, the five named subspecific taxa of *S. rubra* are part of a polytomy without discernable structure. This study suggests that *S. purpurea* ssp. *purpurea* var. *burkii* (which has been named a separate species as *S. rosea*) may be considered a distinct species. If so treated, then the number of species of *Sarracenia* stands at nine.

Key Words: Darlingtonia, Sarracenia, Heliamphora.

The Sarraceniaceae are a small family of insectivorous herbs native to North and South America. The family is composed of three geographically separated genera. *Heliamphora* consists of about six species and occurs in Venezuela and British Guiana (Lloyd 1942; DeBuhr 1975; Maguire 1970, 1978). The eight or so species of *Sarracenia* occur in the southeastern US (Lloyd 1942; McDaniel 1966; DeBuhr 1975) with one, *S. purpurea*, ranging as far north as Canada (Maguire 1970). The monotypic *Darlingtonia* occurs in northern California and western Oregon (Lloyd 1942; DeBuhr 1975).

Infrafamilial Relationships

Several hypotheses concerning the infrafamilial relationships of *Sarraeniaceae* have been offered. Croizat (1960) suggested that the ancestor to Sarraceniaceae may have arrived in South America via Africa. His hypothesis is consistent with positioning the South American *Heliamphora* as sister to a *Sarracenia-Darlingtonia* clade. McDaniel (1966) suggested that the ancestral Sarraceniaceae had begun migrations into their present locations during the pre-Cretaceous. In support of his hypothesis, McDaniel (1966) noted that *Sarracenia* and *Heliamphora* occur in areas known for their endemism and antiquity (i.e., Southern Appalachians and Guyana Highlands) respectively.

Thanikaimoni and Vasanthy (1972) performed a palynological study of the Sarraceniaceae and determined that *Heliamphora* has 3-colporate pollen, whereas *Sarracenia* has 9-colporate pollen. Thanikaimoni and Vasanthy (1972) stated that if this character has phylogenetic value, then this finding suggests that *Heliamphora* is more primitive than *Sarracenia*.

Maguire (1978) suggested that the profound morphological distinctions among the three genera indicate an ancient independent history for each genus in the family. He further suggested that morphological characters suggest that *Heliamphora* is closest to any ancestral prototype and that the origin of this ancestor was in the Guyana Highlands of South America.

Mellichamp (1983) posited that ancestral pitcher plants evolved approximately 40–60 MYA in what is now the southeastern United States. At that time, the climate was favorable to pitcher plants and may have allowed *Darlingtonia* or its ancestors to migrate across the continent to the west coast before the rise of the Sierras and the Rocky Mountains, and allowed *Heliamphora* to migrate to South America (Mellichamp 1983).

According to Renner (1989), *Sarracenia* and *Heliamphora* probably arose from ancestral stock that was widespread and adapted to acidic bogs.

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During severe climactic change (such as that which occurred in the Pleistocene) these bog habitats were largely lost. The remaining bog habitats were isolated and the surviving plants there became specialized in their floral biology (Renner 1989).

Results from a phylogenetic analysis based on chloroplast *rbc*L sequences by Albert et al. (1992) suggested that the African taxon *Roridula* (Roridulaceae) is sister to Sarraceniaceae. That study also indicated that *Darlingtonia* is sister to a *Heliamphora-Sarracenia* clade. Albert et al.'s (1992) phylogeny is supported by the molecular-based study by Bayer et al. (1996) in which both *rbc*L and Internally Transcribed Spacer (ITS) 1 and 2 DNA sequences were combined in one analysis. That study also found that *Roridula* is sister to Sarraceniaceae and that *Darlingtonia* is sister to a *Heliamphora-Sarracenia* clade.

Infrageneric Relationships of *Sarracenia*

The systematic relationships within the genus *Sarracenia* are uncertain and there is no consensus on the number of species within the genus. In a taxonomic revision of *Sarracenia*, McDaniel (1966) reported a characteristic pattern of flavonoids for each species of *Sarracenia*. However, he concluded that these data provided little evidence of phylogenetic relationships.

Schnell and Krider (1976) performed a phenetic analysis of the genus using 19 traditional (non-molecular) characters. The dendrogram produced from that analysis consisted of four main operational taxonomic unit clusters. However, such an analysis, based on overall similarity, cannot be used to infer phylogenetic relationships.

An analysis by Romeo et al. (1977) concluded that the flavonoid components of all *Sarracenia* species are remarkably similar. However, they noted that a consistent lack of certain components in the "rubra-complex" (*sensu* Case and Case 1976) suggested that this closely related group may be derived within the genus. Additionally, they noted that the northern populations of *S. purpurea* but not the southern populations lack these same two components. Romeo et al. (1977) attributed this difference to the recent availability of northern habitats for *S. purpurea*. In contrast to the findings by McDaniel (1966), Romeo et al. (1977) found no characteristic pattern of flavonoids for each species of *Sarracenia*.

Schnell (1978) performed a chromatographic study of petal extract of *Sarracenia*. Although he discussed possible phylogenetic relationships among the representatives sampled, Schnell (1978) concluded that those data are of limited value in that regard. Finally, the study based on combined ITS 1 and 2 DNA sequences by Bayer et al. (1996) recovered trees with little resolution and poor bootstrap support with respect to relationships of *Sarracenia*.

Infraspecific Relationships of *Sarracenia rubra*

Much discussion concerning the variants of *Sarracenia rubra* is present in the literature. For example, McDaniel (1966) noted that *S. rubra* occurs in isolated areas from Mississippi to North Carolina and that various populations of this species may have been separated for longer periods than disjunct populations of other species of *Sarracenia*. McDaniel (1966) further noted that mountain populations are morphologically different but connected by intermediates to sandhill area populations. Individuals from the outer coastal plain of the Carolinas and from west Florida to Mississippi are the most diverged (McDaniel 1966). Later, McDaniel (1971) stated that *Sarracenia rubra* has four morphological forms that are correlated with geographical distribution. McDaniel (1971) noted that intergradation between these forms is common and that the naming of infraspecific taxa is not warranted.

However, other authors have recognized and named variants of *S. rubra*. For example, Wherry (1929) named the disjunct mountain variant as the new species *S. jonesii* but subsequently reduced its rank to *S. rubra* ssp. *jonesii* (Wherry 1933). Case and Case (1974) named the central Alabama disjunct as the new species *Sarracenia alabamensis*.

The naming of variants of *S. rubra* is supported by a morphology-based phenetic analysis of *Sarracenia* by Schnell and Krider (1976). In that study, the authors concluded that the degree of dissimilarity among the mountain, Gulf Coast and eastern Carolina populations would indicate that some infraspecific taxonomic separation may be warranted.

Schnell (1977) stated that there is insufficient discontinuity of characters among the variants of *S. rubra* to consider any as distinct species. However, he did note that as many as five subspecies may be recognized. Therefore, Schnell (1977) reduced the rank of *S. alabamensis* to *S. rubra* ssp. *alabamensis* and named the populations from southern Alabama as *S. rubra* ssp. *wherryi*. Later, Schnell (1979a) named the populations from northwest Florida as *S. rubra* ssp. *gulfensis*.

The molecular-based study by Bayer et al. (1996) failed to resolve the relationship between two *S, rubra* variants they termed S. *rubra* and S. *jonesii*.

Infraspecific Relationships of *Sarracenia purpurea*

Much literature also has been devoted to the variants of *S. purpurea*. Rafinesque (1840) recognized two morphologically and geographically

distinct taxa. The long, glabrous-leaved northern species, occurring from Canada to Virginia, was named *Sarazina* (=*Sarracenia*) *gibbosa* and the short, pubescent variant, occurring from Virginia to Florida, was named *Sarazina venosa*. Rafinesque (1840) also recognized *Sarazina heterophylla* from New England. Wherry (1933) renamed and reduced the rank of Rafinesque's (1840) northern species to *Sarracenia purpurea* ssp. *gibbosa* and the southern species to *S. purpurea* ssp. *purpurea*. Because the ranges of the two subspecies overlap and intergradation occurs in southern New Jersey (Wherry 1933), Wherry (1973) reaffirmed segregating the two variants into subspecies (rather than species). Godt and Hamrick (1998), however, reported that the ranges of the two subspecies overlap in Maryland and Delaware; according to Kartesz and Meacham (1999) both subspecies occur in Delaware, New Jersey and Virginia.

The flavonoid- and amino acid-based study of Romeo et al. (1977) suggested a distinction between the northern and southern populations of *S. purpurea*. Specifically, although they found that the major flavonoid components of all *Sarracenia* species were remarkably similar, they noted that two flavonoid components were absent in the northern populations of *S. purpurea* whereas, they were present in the southern populations.

A petal extract chromotography study by Schnell (1978) failed to find a material distinction between the two subspecies of *Sarracenia purpurea*. However, due to the limitations of this technique in *Sarracenia*, Schnell (1978) suggested that his results did not necessarily discount the recognition of subspecific status for each.

Schnell (1979b) noted that clinal, genetic and phenotypic variations are to be expected in populations of *S. purpurea* due to its extensive range. Although he cautiously accepted the two subspecies named by Wherry (1933), he thought there was little basis for the naming of variants within *S. purpurea* ssp. *venosa*. However, Schnell (1979b) mentioned that additional research may warrant the naming of a new variety for the Gulf Coast populations. Later, Schnell (1993) named the Gulf Coast populations *S. purpurea* ssp. *venosa* var. *burkii*. based on an analysis of morphological characters. Naczi et al. (1999) elevated this taxon to specific status as *S. rosea*. Schnell and Determann (1997) recognized another southern variant native to the mountains and Piedmont of Georgia and North Carolina and named it *S. purpurea* ssp. *venosa* var. *montana*.

In their phenetic analysis, Godt and Hamrick (1998) reported that their most striking observation is the high level of allozyme divergence found among *S. purpurea* populations. They stated that 90% of this divergence is due to differences between infraspecific taxa and suggested that this divergence is due primarily to restricted gene exchange for a considerable period of time. Their phenogram indicates that the Gulf Coast populations (var. *burkii*) are the most distinct, that the Atlantic Coast populations (var. *venosa*) are most closely allied with the mountain populations (var. *montana*) followed by the northern populations (ssp. *purpurea*).

In contrast to the high level of allozyme divergence found in the *S. purpurea* species complex, Godt and Hamrick (1998) reported that there is little genetic differentiation among disjunct subspecies of the *S. rubra* complex. This suggests that the *S. rubra* subspecies diverged rather recently or that levels of gene flow between them have been high (Godt and Hamrick 1998).

Ellison et al. (2004) reported that morphological variation in *Sarracenia purpurea* is associated with environmental factors and geography. Specifically, they indicated that the size and shape of pitchers are primarily a function of precipitation, temperature and latitude. Ellison et al. (2004) reported that there is no obvious way to distinguish the two subspecies of *S. purpurea* by morphology and that this supports Gleason and Cronquist (1991) in that the two subspecies are merely geographical variants. However, Ellison et al. (2004) claimed that their data do support the differentiation of the Gulf Coast populations.

The goal of this study is to develop a molecular-based phylogeny of the Sarraceniaceae with primary interest on the genus *Sarracenia*. This phylogeny, will be inferred from combined nuclear-encoded ITS2 and 26S large ribosomal subunit rRNA gene sequences. A well-supported phylogeny will provide additional insight into the evolutionary patterns and relationships that will serve as a basis for comparison with previous studies.

Methods

Vouchers and GenBank accessions for the taxa included in this study are listed in Table 1. The ingroup consists of representatives from *Sarracenia, Heliamphora*, and *Darlingtonia* (Table 1). *Roridula* was selected as outgroup following Albert et al. (1992) and Bayer et al. (1996). All taxa included in *Sarracenia* (*sensu* Kartesz and Meacham 1999) are included. This includes all subspecific taxa within the *S. rubra* and *S. purpurea* complexes. Note that Kartesz and Meacham (1999) include southern populations of *S. purpurea* in ssp. *purpurea* (not ssp. *venosa*) and northern populations in ssp. *gibbosa*.

For enhanced context, multiple representatives of *Sarracenia alata* and *S. leucophylla* are included. Although the range of *S. alata* is separated into an eastern and western disjunct (Sheridan 1991), no infraspecific taxa have been named. Three representatives from each disjunct

TABLE 1. TAXA ANALYZED IN THIS STUDY. All ingroup representatives are from Sarraceniaceae with *Roridula dentata* (Roridulaceae) as outgroup. All sequences have been deposited in GenBank. Vouchers are housed at the McNeese State University herbarium (MCN). Location data for wild-collected specimens are indicated. Taxonomy follows Kartesz and Meacham (1999). Representatives of *Sarracenia alata* from the eastern and western disjuncts are designated.

Taxon	Voucher	GenBank accession
Sarracenia alata Wood (Western Disjunct)	Neyland 1496 Calcasieu Parish, LA	AY795884
Sarracenia alata Wood (Eastern Disjunct)	Neyland 2112 Jackson County, MS	AY789969
Sarracenia alata Wood (Eastern Disjunct)	Neyland 2120 Tangipahoa Parish, LA	AY789968
Sarracenia alata Wood (Eastern Disjunct)	Neyland 2108 Stone County, MS	AY795883
Sarracenia alata Wood (Western Disjunct)	Neyland 2122 Hardin County, TX	AY796054
Sarracenia alata Wood (Western Disjunct)	Neyland 2123 Natchitoches Parish, LA	AY795885
Sarracenia flava L.	Neyland 2109 Santa Rosa County, FL	DQ017391
Sarracenia leucophylla Raf.	Neyland 2113 Jackson County, MS	AY796055
Sarracenia leucophylla Raf.	Neyland 2110 Santa Rosa County, FL	DQ088065
Sarracenia leucophylla Raf.	Neyland 2117 Baldwin County, AL	DQ088066
Sarracenia minor Walt.	Neyland 2139	DQ073470
Sarracenia oreophila (Kearney) Wherry	Neyland 2131	AY950690
Sarracenia psittacina Michx.	Neyland 2121 Tangipahoa Parish, LA	AY967802
Sarracenia purpurea L. ssp. *gibbosa* (Raf.) Wherry	Neyland 2137	DQ028630
Sarracenia purpurea L. ssp. *purpurea* var. *burkii* Schnell	Neyland 2142 Escambia County, FL	DQ088067
Sarracenia purpurea L. ssp. *purpurea* var. *montana* Schnell & Determann	Neyland 2136 Henderson County, NC	DQ028631
Sarracenia purpurea L. ssp. *purpurea* var. *purpurea*	Neyland 2154	DQ098117
Sarracenia rubra Walt. ssp. *alabamensis* (F.W. & R.B. Case) Schnell	Neyland 2129	AY942694
Sarracenia rubra Walt. ssp. *gulfensis* Schnell	Neyland 2141	DQ076326
Sarracenia rubra Walt. ssp. *jonesii* (Wherry) Wherry	Neyland 2130 Henderson County, NC	DQ017392
Sarracenia rubra Walt. ssp *rubra*	Neyland 2135	DQ028629
Sarracenia rubra Walt. ssp. *wherryi* (F.W. & R.B. Case) Schnell	Neyland 2153	DQ076326
Darlingtonia californica Torr.	Neyland 2133	DQ017390
Heliamphora heterodoxa Steyerm.	Neyland 1809	AY796056
Roridula dentata L	Neyland 2128	AY950689

were included in this analysis. The recovered systematic patterns among the disjunct representatives of *S. alata* are compared with those among the disjunct representatives of both *S. rubra* and *S. purpurea*.

The range of *S. leucophylla* is continuous and no infraspecific taxa have been named. Three representatives from different populations of *S. leucophylla* were also included in this analysis. The recovered systematic patterns among these three *S. leucophylla* representatives are, likewise, compared with those among the disjunct representatives of *S. rubra, S. purpurea*, and *S. alata*.

When possible, DNA was extracted from the leaves of plants in natural populations. However, in some cases, leaves from greenhouse maintained individuals were used. Collection details for samples are referenced in Table 1.

An approximate 1kb DNA segment of the 26S gene and an approximate 245 base-pair length nuclear ribosomal ITS2 region for each representative listed in Table 1 was analyzed in this study. Because Bayer et al. (1996) used ITS sequences with limited success, the 26S segment was sequenced to augment the amount of data for this analysis. The ITS2 and 26S segments are contiguous in the nuclear genome. The 26S fragment which spans base positions 1–958 in *Nicotiana tabacum* (GenBank Accession AF479172) is characterized by conserved segments and more variable expansion segments designated as D2, D3, and D4 by Kuzoff et al. (1998). Most of the variability within this gene is found in the first kb (Kuzoff et al. 1998). The rate of divergence in this 26S segment has been shown to be informative at the specific and infraspecific level in studies of the family Ericaceae which is closely related to Sarraceniaceae (e.g., Neyland 2004; Neyland and Hennigan 2004).

DNA sequences were used to infer systematic relationships of *Sarraceniaceae* through a maximum parsimony phylogenetic analysis using the heuristic search algorithm with Phylogenetic Analysis Using Parsimony (PAUP version 4.0b10) software (Swofford 2002). Searches employed 1000 random stepwise addition replications. All characters including transitions and transversions were weighted equally. Gaps were treated as missing data. Disk copies of aligned sequences are available from the author. As a measure of clade stability or robustness, bootstrap support (Felsenstein 1985) was calculated. Ten thousand bootstrap replications were employed in this analysis (MulTrees option in effect).

Total DNA was extracted from tissue using the CTAB method of Doyle and Doyle (1987). DNA sequences were amplified via polymerase chain reaction (PCR) (Mullis and Faloona 1987) with combinations of forward and reverse primers referenced in Neyland (2002).

DNA was amplified with Tfl enzyme (Epicentre Technologies, Madison, WI), using the following thermocycling protocol: a hot start at 94°C for 3 min; 30 amplification cycles of 94°C for 1 min, 55°C for 1 min; 72°C for 3.5 min, a terminal extension phase at 72°C and an indefinite terminal hold at 4°C. The double-stranded PCR product was purified with QIAquick (Qiagen, Hilden, Germany) using the manufacturer's protocol. Two µl of each sample was electrophoresed in a 1.0% agarose mini-gel for quantification against a known standard. Automated sequencing was conducted on an ABI Prism 377 Sequencer with XL Upgrade (housed at Louisiana State University, Baton Rouge, LA, USA) using ABI Prism, Big Dye Terminator cycle sequencing protocol (P.E. Applied Biosystems, Foster City, CA, USA). Sequences have been deposited in the GenBank database (Table 1).

Results

Sequences were aligned by visual inspection. Gaps were introduced to accommodate 29 single-point insertions/deletions (INDELS) in the data set. Nineteen gaps were inserted in the ITS2 segment and 10 gaps were inserted in the 26S segment. INDELS were not treated as characters. The largest absolute distance between any two members in the data set was 134 between *Roridula dentata* and *Sarracenia minor*. The smallest absolute distance between any two members in the data set was 0 between *Sarracenia alata* (2108) and *S. alata* (2112); *S. alata* (2123) and *S. alata* (2122); *S. leucophylla* (2110) and *S. leucophylla* (2117); *S. rubra* ssp. *wherryi* and *S. oreophila; S. rubra* ssp. *jonesii* and *S. oreophila*. Unambiguous transitions and transversions numbered 116 and 43 respectively. Therefore, transitions outnumbered transversions by a factor of about 3 to 1. Phylogenetic analyisis resulted in the recovery of 51 most-parsimonious trees. Each tree was 279 steps with a consistency index of 0.9068 and a retention index of 0.8497.

Systematic Relationships of Sarraceniaceae

As depicted in the cladograms, *Darlingtonia* is sister to a *Heliamphora-Sarracenia* clade (Figs. 1, 2). This branching pattern is consistent with the molecular-based phylogenies recovered by Albert et al. (1992) and Bayer et al. (1996). The place of origin for the ancestral Sarraceniaceae is equivocal.

Systematic Relationships of *Sarracenia*

The recovered topology strongly supports the position of *Sarracenia purpurea* as sister to the remaining species of the genus (Figs. 1, 2). This position is contrary to that suggested by Bayer et al.'s (1996) study that indicated that *S. alata* is sister to all other species in the family and that *S. purpurea* is sister to *S. leucophylla*. However, the branches that depicted those relationships in Bayer et al.'s (1996) study received less than 50% bootstrap support. Additionally, the findings of the present study do not support the cluster composed of *S. purpurea, S. leucophylla*, and *S. psittacina* recovered by Schnell and Krider's (1976) phenetic analysis.

All four named infraspecific taxa within *Sarracenia purpurea* were resolved (Figs. 1, 2). Absolute nucleotide pair-wise differences among these taxa range from 4 to 10. The cladistic relationships among these taxa in this study match the distance relationships reported by Godt and Hamrick (1998). The branching pattern in this clade depicts *S. purpurea* ssp. *purpurea* var. *burkii* as sister to the remaining infraspecific taxa.

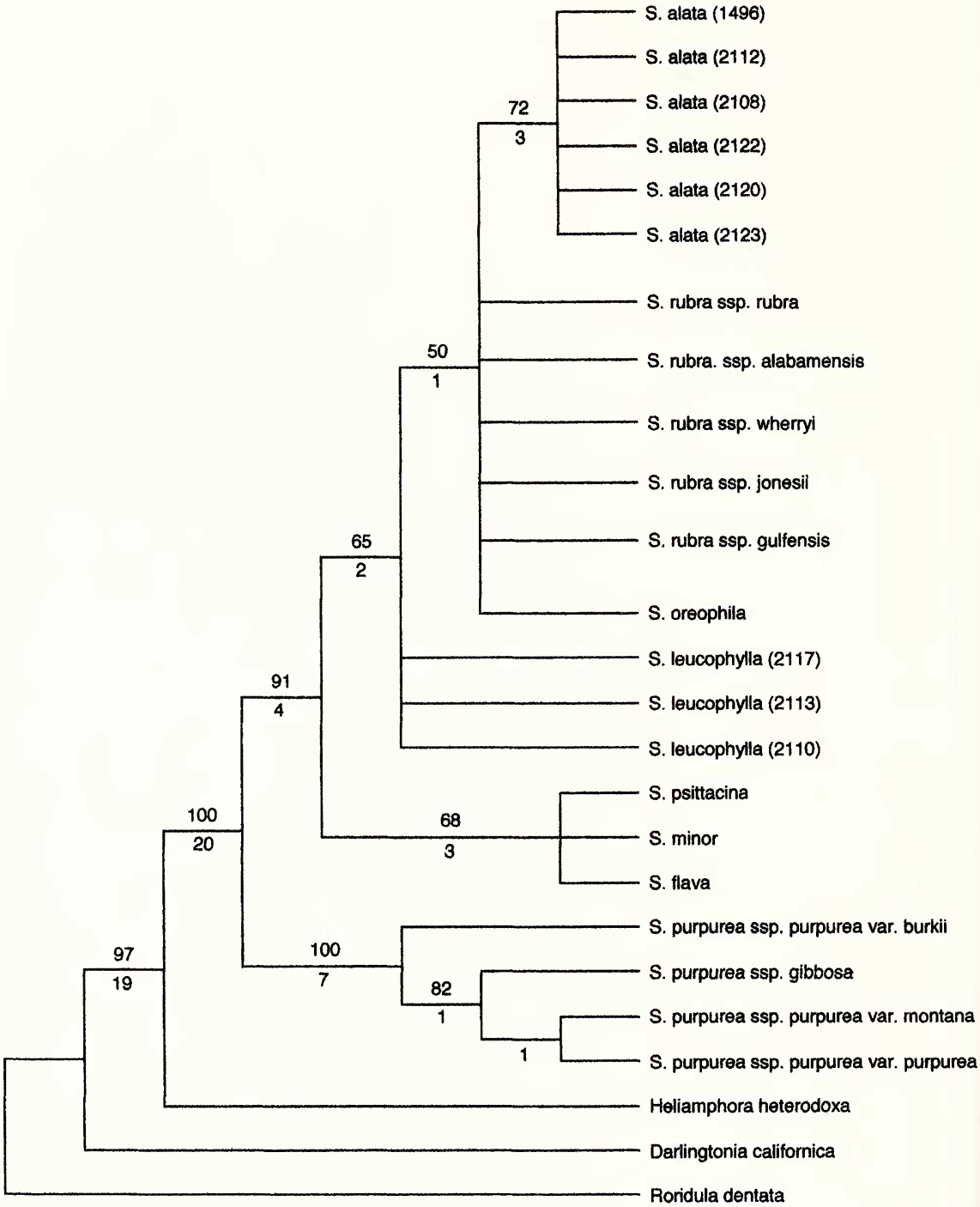

FIG. 1. Strict consensus tree recovered from a maximum parsimony heuristic search. Bootstrap values greater than 50% are indicated above each branch. Unequivocal synapomorphies are indicated below each branch. Voucher numbers for taxa with multiple representatives are indicated.

Therefore, the recovered topology suggests that *S. purpurea* ssp. *purpurea* is polyphyletic. Furthermore, the strongly supported dichotomy between *S. purpurea* ssp. *purpurea* var. *burkii* and the other infraspecific *S. purpurea* supports Naczi et al.'s (1999) elevation of this taxon to specific status as *Sarracenia rosea*.

The recovered topology suggested a moderately supported clade composed of *Sarracenia flava, S. minor*, and *S. psittacina* (Figs. 1, 2). This same

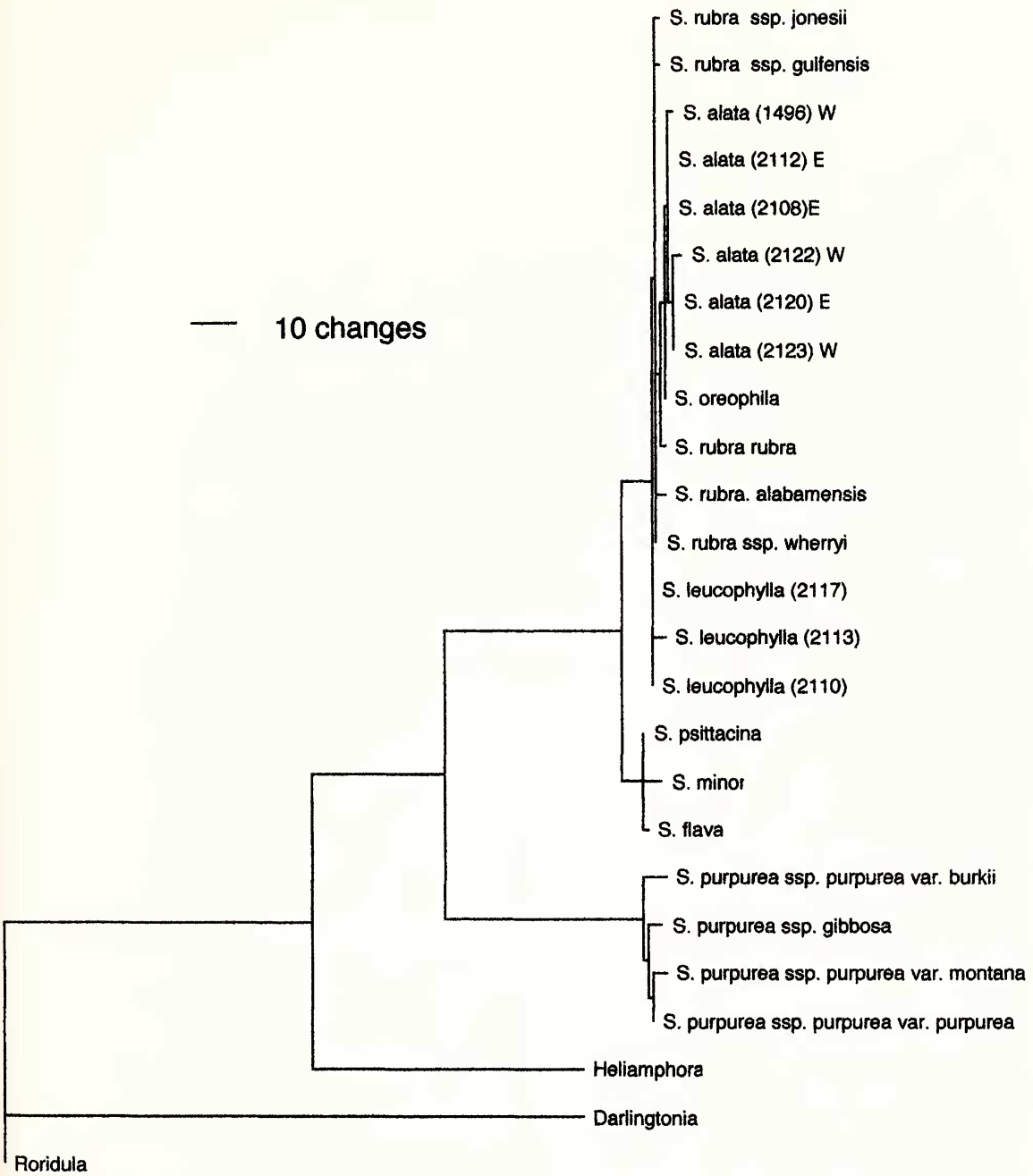

FIG. 2. Phylogram of one of the 51 most-parsimonious trees recovered in the maximum parsimony heuristic search. The changes legend indicates branch length.

clade was recovered in Bayer et al.'s (1996) analysis and was the only clade within *Sarracenia* that received greater than 50% bootstrap support in that study.

The polytomy consisting of *Sarracenia oreophila*, six representatives of *S. alata* and the five subspecies of *S. rubra* was moderately supported and suggests a close affinity among these three species. Because there were no more than two absolute nucleotide differences between any two representatives, the subspecies of *S. rubra* appeared to be very closely related.

Although the eastern and western disjuncts of *Sarracenia alata* are separated by over 300 km at their closest point, the recovered topology suggests no discernable phylogenetic structure between the two (Figs. 1, 2). No more than three absolute nucleotide differences between the se-

quences of any two representatives were evident in the data. The derived position of *S. alata* contrasts with its basal position recovered in the topology by Bayer et al. (1996). However, as previously stated, the branch supporting the position of *S. alata* in the Bayer et al. (1996) study received less than 50% bootstrap support. The close affinity between *S. alata* and *S, rubra* has been suggested previously (McDaniel 1966; Schnell and Krider 1976).

Sarracenia oreophila also appeared in the derived polytomy with *S. rubra* and *S. alata*. Absolute nucleotide pair-wise differences between *S. oreophila* and the representatives from *S. rubra* and *S. alata* ranged between 0 and 2. An affinity between *S. oreophila* and S. *alata* has been suggested (McDaniel 1966; Schnell 1979b) and an affinity between *S. oreophila* and *S. rubra* has been suggested (McDaniel 1966; Case and Case 1976). However, *S. oreophila* clustered with *S. flava* in the phenetic study of Schnell and Krider (1976) and its position was unresolved in Bayer et al.'s (1996) study. The derived polytomy consisting of *S. alata, S. rubra* and *S. oreophila* suggests that these taxa are closely related, have evolved relatively recently, and have radiated rapidly.

The three representatives of *Sarracenia leucophylla* appeared in the cladogram as sister to the polytomy that includes *S. alata, S. oreophila*, and *S. rubra*. Absolute nucleotide differences among the representatives of *S. leucophylla* numbered no more than three. Therefore, the absolute nucleotide differences among representatives of *S. leucophylla* (a species with a continuous range) were comparable with *S. alata* and *S. rubra* (two species with disjunct ranges). No branches supporting infraspecific relationships were recovered in the strict consensus tree for any of these three species. One interpretation of this finding is that present disjunct populations have been founded only recently (cf. Godt and Hamrick 1998).

Discussion

The place of origin for the ancestral Sarraceniaceae is equivocal. However, with *Darlingtonia's* position in the cladograms (Figs. 1, 2), it appears that a subtropical North American origin is at least as likely as a Neotropical one for the family. As suggested by Bayer et al. (1996), if Sarraceniaceae originated in subtropical North America, then *Heliamphora* may have originated by a single long-distant dispersal event. However, if Sarraceniaceae had a Neotropical origin, then two dispersal events may have occurred to account for the present distribution (Bayer et al. 1996). Because other previously mentioned scenarios are also possible (cf. Croizat 1960; McDaniel 1966; Mellichamp 1983; Renner 1989), the origin and migration of ancestral Sarraceniaceae remain unresolved.

Although all relationships have not been resolved, the present analysis brings new insight into the evolution of *Sarracenia*. One of the major findings of this study, is that *S. purpurea* is sister to all remaining species in *Sarracenia* and that a major subsequent dichotomy in the evolution of the genus has resulted in one clade composed of *S. minor, S. psittacina*, and *S. flava* and second clade composed of *S. alata, S. rubra, S. oreophila*, and *S. leucophylla*.

Another major finding of this research is that the named subspecies of *Sarracenia rubra* do not appear in a discernable phylogenetic structure (Figs. 1, 2). The hypothesis by Romeo et al. (1977) that the *S. rubra* complex is derived within the genus was supported by the complex's position in the cladograms (Figs. 1, 2). Although the representatives of *S. alata* were moderately supported as a monophyletic group, the systematic relationships among the five subspecies of *S. rubra* were unresolved. Similarly, Bayer et al.'s (1996) analysis failed to resolve the relationship between *S. rubra* and *S. jonesii*.

Therefore, although each subspecies may be disjunct and exhibit minor morphological differences, the naming of *S. rubra* subspecies may be tenuous. These findings support McDaniel's (1971) contention that the naming of infraspecific taxa of *S. rubra* is not warranted. Additionally, the naming of *S. jonesii* and *S. alabamensis* as separate species is not supported by this study. Although there is little molecular distinction between *S. oreophila* and the representatives of *S. rubra*, the two taxa are morphologically distinct and may be considered separate species, at least by the criteria embodied in the morphospecies concept.

In contrast, representatives from the named infraspecific taxa of *S. purpurea* appear in a resolved clade (Figs. 1, 2). If *S. purpurea* ssp. *purpurea* var. *burkii* is treated as a distinct species (i.e., *S. rosea*), then the number of species in the genus stands at nine. By this logic, each of the remaining three infraspecific taxa could also be named as distinct species which would increase the number of species in the genus to twelve.

An additional finding of this research concerns the putative affinity among *Sarracenia psittacina, S. flava* and *S. minor*. As noted previously, this same clade was recovered in Bayer et al.'s (1996) analysis. However, other studies have suggested different affinities for these three taxa. For example, *S. psittacina* has been aligned with *S. purpurea* (McDaniel 1966; Schnell and Krider 1976). Suggested affinities for *S. flava* include *S. leucophylla* (McDaniel 1966), *S. oreophila* (Schnell and Krider 1976; Schnell 1978) and *S. alata* (McDaniel 1966; Schnell 1978). MacFarlane (1893) considered *S. minor* to be similar to

the ancestral form of *Sarracenia* and, therefore, it would occupy the basal position in the genus. McDaniel (1966) suggested that *S. minor* has a close affinity with *S. rubra*. In the phenetic study by Schnell and Krider (1976), *S. minor* appeared isolated and clustered with no other taxa.

Future research aiming to clarify these remaining unresolved relationships within *Sarracenia* must employ DNA sequences with very high mutation rates. Although the ITS regions and the first kb of the 26S gene exhibit comparatively high mutation rates, it is apparent that more informative characters will be necessary to bring a higher degree of resolution to the genus. However, it is unclear what other sequence fragments may be useful in this regard. Future research efforts may resolve, for example, the relationships among the morphologically distinct *S. alata, S. rubra*, and *S. oreophila*. The resolution of systematic relationships among the morphologically similar subspecies of *S. rubra* may prove more problematic.

The recovery of a completely resolved and robustly supported phylogeny of *Sarracenia* remains elusive. Perhaps the problem was described best by Schnell and Krider (1976) who stated that the genus is probably incompletely differentiated with all species very closely related in a genetic and evolutionary sense. In a genus that easily produces natural hybrids (cf. DeBuhr 1975; Schnell and Krider 1976), there is a distinct possibility that several recognized species of *Sarracenia* have arisen through hybridization and introgression (cf. Anderson 1953; Stebbins 1959; Riesenberg and Eilstrand 1993; Arnold and Hedges 1995; McDade 1995; Bayer et al. 1996; Ellison 2004). Such processes result in reticulate evolutionary patterns that are difficult to decipher.

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Literature Cited

Anderson, E. 1953. Introgressive hybridization. Biological Reviews 28:280–307.

Albert, V. A., S. E. Williams, and M. W. Chase. 1992. Carnivorous plants: phylogeny and structural evolution. Science 257:1491–1495.

Arnold, J. and S. A. Hedges. 1995. Are natural hybrids fit or unfit relative to their parents? Trends in Ecology and Evolution 10:67–71.

Bayer, R. J., L. Hufford, and D. E. Soltis. 1996. Phylogenetic relationships in Sarraceniaceae based on *rbc*L and ITS sequences. Systematic Botany 21:121–134.

Case, F. W. and R. B. Case. 1974. *Sarracenia alabamensis*, a newly recognized species from central Alabama. Rhodora 76:650–665.

——— and ———. 1976. The *Sarracenia rubra* complex. Rhodora 78:270–325.

Croizat, L. 1960. Principia botanica. Published by the author, Caracas, Venezuela.

DeBuhr, L. E. 1975. Phylogenetic relaionships of the Sarraceniaceae. Taxon 24:297–306.

Doyle, J. J. and J. L. Doyle. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochemical Bulletin 19:11–15.

Ellison, A. M., H. L. Buckley, T. E. Miller, and N. J. Gotelli. 2004. Morphological variation in *Sarracenia purpurea* (Sarraceniaceae): geographic, environmental, and taxonomic correlates. American Journal of Botany 91:1930–1935.

Felsenstein, J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39:783–791.

Gleason, H. A. and A. Cronquist. 1991. Manual of vascular plants of northeastern United States and adjacent Canada. New York Botanical Garden, Bronx, NY.

Godt, M. J. and J. L. Hamrick. 1998. Genetic divergence among infraspecific taxa of *Sarracenia purpurea*. Systematic Botany 23:427–438.

Kartesz, J. T. and C. A. Meacham. 1999. Synthesis of the North American flora. North Carolina Botanical Garden, Chapel Hill, NC.

Kuzoff, R. K., J. A. Sweere, D. E. Soltis, P. S. Soltis, and E. A. Zimmer. 1998. The phylogenetic potential of entire 26S rDNA sequences in plants. Molecular Biology and Evolution 15:251–263.

Lloyd, F. 1942. The carnivorous plants. Chronica Botanica, Waltham, MA.

MacFarlane, J. M. 1893. Observations on pitchered insectivorous plants II. Histology of *Darlingtonia, Sarracenia* and *Heliamphora* with remarks on adaptations for insect-catching. Annals of Botany 7:403–458.

Maguire, B. 1970. On the flora of the Guyana Highland. Biotropica 2:85–100.

———. 1978. Botany of the Guyana Highland: Sarraceniaceae. Memories of the New York Botanical Garden 29:36–62.

McDade, L. A. 1995. Hybridization and phylogenetics. Pp. 305–331 *in* P. C. Hoch and A. G. Stephenson (eds.), Experimental and molecular approaches to plant biosystematics. Missouri Botanical Garden, St. Louis MO.

McDaniel, S. 1966. A taxonomic revision of *Sarracenia* (Sarraceniaceae). Ph.D. dissertation. Florida State University, Tallahassee, FL.

———. 1971. The genus *Sarracenia* (Sarraceniaceae). Bulletin of the Tall Timbers Research Station 9:1–36.

Mellichamp, T. L. 1983. Cobras of the Pacific Northwest. Natural History 4:47–51.

Mullis, K. B. and F. A. Faloona. 1987. Specific synthesis of DNA in vitro via polymerase chain reaction. Methods in Enzymology 155:335–350.

Naczi, R. W. C., E. M. Soper, F. W. Case Jr., and R. B. Case. 1999. *Sarracenia rosea* (Sarraceniaceae), a new species of pitcher plant from the southeastern United States. Sida 18:1183–1206.

NEYLAND, R. 2002. A phylogeny inferred from large ribosomal subunit 26S rDNA gene sequences suggests that Burmanniales are polyphyletic. Australian Systematic Botany 15:1–10.

———. 2004. The systematic significance of color variation in *Monotropa hypopithys* (Ericaceae) inferred from large ribosomal subunit (26S) rRNA gene sequences. Madroño 51:275–279.

——— AND M. HENNIGAN. 2004. Systematic relationships of *Monotropa uniflora* (Ericaceae) inferred from large ribosomal subunit (26S) rRNA gene sequences. Castanea 69:265–271.

RAFINESQUE, C. S. 1840. Autikon Botanikon. Philadelphia.

RENNER, S. S. 1989. Floral biological observations on *Heliamphora tatei* (Sarraceniaceae) and other plants from Cerro de la Neblina in Venezuela. Plant Systematics and Evolution 163:21–29.

RIESENBERG, L. H. AND N. C. EILSTRAND. 1993. What can molecular and morphological markers tell us about plant hybridization? Critical Reviews in Plant Science 12:213–241.

ROMEO, J. T., J. D. BACON, AND T. J. MABRY. 1977. Ecological considerations of amino acids and flavonoids in *Sarracenia* species. Biochemical Systematics and Ecology 5:117–120.

SCHNELL, D. E. 1977. Infraspecific variation in *Sarracenia rubra* Walt.: some observations. Castanea 42:149–170.

———. 1978. *Sarracenia* L. Petal extract chromatography. Castanea 43:107–115.

———. 1979a. *Sarracenia rubra* Walter ssp. *Gulfensis*: a new subspecies. Castanea 44:218–219.

———. 1979b. A critical review of published variants of *Sarracenia purpurea* L. Castanea 44:47–59.

———. 1993. *Sarracenia purpurea* L. ssp. *venosa* (Raf.) Wherry var. *burkii* Schnell (Sarraceniaceae): a new variety of the Gulf coastal plain. Rhodora 95:6–10.

——— AND D. W. KRIDER. 1976. Cluster analysis of the genus *Sarracenia* L. in the southeastern United States. Castanea 41:165–176.

——— AND R. O. DETERMANN. 1997. *Sarracenia purpurea* L. ssp. *venosa* (Raf.) Wherry var. *montana* Schnell & Determann (Sarraceniaceae): a new variety. Castanea 62:60–62.

SHERIDAN, P. M. 1991. What is the identity of the of the West Gulf Coast pitcher plant *Sarracenia alata* Wood? Carnivorous Plant Newsletter 20:102–109.

STEBBINS, G. L. 1959. The role of hybridization in evolution. Proceedings of the American Philosophical Society 103:231–251.

SWOFFORD, D. L. 2002. PAUP: phylogenetic analysis using parsimony. Version 4.0b10. Sinauer Associates, Sunderland, MA.

THANIKAIMONI, G. AND G. VASANTHY. 1972. Sarraceniaceae: palynology and systematics. Pollen et Spores 14:143–155.

WHERRY. 1929. Acidity relations of the *Sarracenias*. Journal of the Washington Academy of Sciences. 19:379–390.

———. 1933. The geographic relations of *Sarracenia purpurea*. Bartonia. 15:1–6.

———. 1972. Notes on *Sarracenia* subspecies. Castanea 37:146–147.

———. 1973. Reminiscences on carnivorous plants. Carnivorous Plant Newsletter 2:35–37.